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R. Bias

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Cadmium uptake rates in euryhaline amphipods of the Elbe estuary 84

Experiments with Corophium volutator (PALLAS)(Amphipoda, Corophiidae)[1]

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with 18 figures and 10 tables in the text

1.3 Selection of the toxic contaminant 86

In numerous experiments published in recent years, the high toxicity of the element cadmium and its bioconcentration in aquatic organisms has been demonstrated (BALL 1967; SHUSTER & PRINGLE 1969; GARDNER & YEVICH 1970; BROWN & AHSANULLAH 1971; HABERER & NORMANN 1971; EISLER et al 1972; KARBE 1972; EISLER & GARDNER 1973; HUTCHINSON & CZYRSKA 1974; WESTERNHAGEN & DETHLEFSEN 1975; MARTIN & FLEGAL 1975; REISH et al 1976; GEORGE & COOMBS 1977).

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On the basis of its biochemical behaviour, cadmium ranks next after mercury, copper and lead in order of toxicity (BOWEN 1966). 87
However, due to its retention in biological material cadmium appears to be particularly significant (FLICK et al 1971; FRIBERG et al 1971).

Due to its minimal distribution in the geosphere (GOLDBERG 1965; WEDEPOHL 1970), wide variations in the regional distribution of cadmium in the biosphere are to be expected, with the highest density in regions threatened by industrial encroachment.

Increased industrial use of this element, which is reflected in the cadmium content of fresh sediments (GADOW & SCHÄFER 1973, 1974; FÖRSTNER & REINECK 1974) presents a risk of unknown magnitude for the environment: cadmium is easily mobilized biologically by the processes of sorption and deposition (DE GROOT 1971; FÖRSTNER & MÜLLER 1974; VALIELA et al 1974; HELZ et al 1975; PITA & HYNE 1975; TAN 1976). As a result, cadmium has a long retention time in the hydrosphere (GOLDBERG 1965). Cadmium has virtually no effect on the organic components of sewage waste (BIAS 1973), which are either decomposed or de-activated by the toxic effects of the majority of heavy metals. The exceptional persistence of cadmium has been mentioned already.

Despite the intensive studies carried out on the problems posed by the toxicity and persistence of cadmium in the environment, numerous questions regarding its accumulation and retention biologically as well as its physiological significance remain unanswered. For these reasons, this element was selected for study. As far as the actual experimentation is concerned, the ease with which cadmium can be identified analytically was convenient.

2.3 Experimental procedure 89

In the laboratory, the Corophium collected at Otterndorf were separated from detritus by submersion in salt water (16% S [salinity]) and subsequent sieving. Covered solid glass tanks with volumes of 1.5, 2.0 and 6.0 L were used as containers for the experiments. Each experiment was carried out in duplicate with 5 cadmium concentrations for a constant salinity (16% S) or at different salinities (4-28% S) for one concentration of the toxic heavy metal.

For all of the experiments with sediments, the ratio of the volume of water to the volume of sediment was 3.6:1.0. Thus for the measurements carried out in the container tanks, the 2.5-3 cm bottom layer was sufficiently deep for the digging activities of the crustaceans. The temperature was maintained at 15°C±1°C, with light and dark phases alternating in a 12-hour pattern.

At least 36 hours adaptation time was allowed when the organisms were transferred to the laboratory. Then the cadmium was administered, always as a freshly-prepared standard solution ($3CdSO_4.8H_2O$).

The experimental conditions were subject to routine monitoring, specifically temperature, oxygen content, pH value, salinity and loss of water as well as spot checks for alkalinity.

During the experiments, at timed intervals water, sediment and organism samples were taken. The crustaceans were removed using a pair of spring-loaded pincers, and then they were transferred to uncontaminated salt water (16‰ S) for two hours in order to eliminate fecal cadmium as well as to rinse off any cadmium clinging to their external surfaces. They were subsequently placed on a sheet of blotting paper for 30 seconds to dry off, and then weighed out into 1.5 ml polypropylene ampoules and fast-frozen (Fig.2).

3.1.1.2 Discussion of cadmium uptake in Corophium 101

The cadmium bioconcentration in these crustaceans proved to be dependent on both the cadmium concentration of the water and its salinity.

For the lower concentration range of 0.01 to 0.3 mg Cd/L, there was a virtually linear relationship between the concentration of the toxic element accumulated in the organisms, and the duration of the experiment over a 92-hour period. GEORGE & COOMBS (1977) also reported a linear bioconcentration function in Mytilus edulis exposed to 0.02 mg Cd/L over a time frame of several days.

For higher concentration ranges, a decrease in the rate of uptake could be observed after the first 24 hours. The accumulation pattern here followed a saturation function, as is reported by other

authors (CEARLEY & COLEMAN 1973; DAVIES 1973; REHWOLDT & KARIMIAN-TEHERANI 1976; KRAMER & NEIDHARDT 1975; ADEMA 1976; see Fig.4).

The results of experiment 2 which extended over a 16-day period indicated that saturation was not reached during the duration of this experiment carried out at higher cadmium concentrations. The cause is possibly an increase in the saturation value as a result of cadmium-induced tissue damage which was observed along with a high mortality 102
rate; another possibility is a distortion in the kinetics because of the unusual vitality of the few surviving organisms. The cadmium content of these surviving crustaceans may not be representative of the collective total of the experimental organisms.

From experiment 3 it can be seen that there is an inverse correlation between the rate of uptake of cadmium and the salinity of the water. For salinities of 4% and 28% the uptake rates were in a ratio of 4:1.

Several authors have observed the dependence of the bioconcentration of heavy metals on the salinity of the water (VERNBERG & VERNBERG 1972a; VERNBERG & O'HARA 1972; OLSEN & HARREL 1973; WESTERNHAGEN et al 1974, 1975; JACKIM et al 1977). As well, in studies on fiddler crabs in winter, O'HARA (1973) observed that the cadmium concentrations of the crustaceans were three times as high for salinities of 10% as for the comparable organisms in salinities of 25%.

There are a variety of possible explanations for the increased bioconcentration of cadmium in water of lower salinity: the cadmium species in water with low salt content may be converted into a more readily assimilated cadmium compound. However, as a rule cadmium is not as readily available as a free ion in fresh water as it is in sea-water (STUMM & MORGAN 1970); GIESY & BRIESE 1978); it is logical therefore to associate the dependence of cadmium bioconcentration on salinity with the variation in the osmotic capability of the organisms at different salt concentrations.

As a euryhaline inhabitant of brackish waters, Corophium volutator possesses the ability to osmoregulate in order to counteract the extreme fluctuations in the salinity of his environment (McLUSKY 1967).

While residing in water with more than 20% salinity, the hemolymph of this crustacean is slightly hypotonic. At salinities lower than 20%, the active osmoregulation begins. For salinities of 2% in the surrounding environment a blood salinity of 13% can still be maintained; for an environmental salinity level of 4%, the corresponding hemolymph salinity is 16% (McLUSKY 1967). The concentration factor in this case is therefore approximately four, compared to only 1.1 for normal marine salinity levels. As far as is currently known, only a few of the essential cations are selectively excepted from the bioconcentration of salts occurring during the course of osmoregulation (BOWEN 1966); these cations are subject to increased secretion. This mechanism is not operative for toxic heavy metals with long biological half-lives. It is therefore likely that the approximately four-fold increase in the bioconcentration of cadmium for organisms in environments of 4% salinity relative to those in 28% salinity corresponds to the increased bioconcentration of salt in the organism, which differs from the level in the external environment by approximately that same factor, four.

3.1.3.2 Analysis of the kinetic relationships in experiments on the bioconcentration of cadmium 121

In the experimental series involving natural sediment from the mud flats, and cadmium administered as a single dose at the beginning of the experiment, it was observed that one main identical accumulation pattern occurred repeatedly in the organisms. Differences in the salinities of the water and variations in the cadmium concentrations of the water-sediment ecosystem affected only the intensity of the bioconcentration process but not the accumulation pattern itself.

Three distinct phases could be distinguished during the period of the bioconcentration (Fig.15):

1. In the first hours of the exposure to cadmium, a high rate of uptake was observed.
2. After 4 to 8, at the maximum 18, days the rate of uptake of cadmium fell off. In a typical case this caused a reduction in the cadmium concentrations of the organisms.
3. After approximately 6 to 14 day duration of the experiments, the cadmium levels again increased. This accelerated over several days and then gradually diminished towards the end of the experiments.

The quartz sand experiments followed this same accumulation pattern 122
except that the phases were considerably easier to identify.

The possible explanations for this accumulation pattern include:
a) physiological reactions of the organism toward the element cadmium;
b) differences based upon the experimental conditions affecting the availability of the cadmium over the course of the experiment.

Re a): A similar accumulation pattern has been observed for algae (HAYWARD 1969; KNAUER & MARTIN 1973; DAVIES 1976; KREMLING et al 1978; M.MELKONIAN & M.WETTERN, personal communication; W.NEUGREVE, personal communication). In all cases the experiments involved constant heavy metal concentrations in a culture medium. There are indications that extra-cellular products affect the heavy metal species and therefore the accumulation pattern as well. Similar interactions have been postulated for fish (WESTERNHAGEN et al 1978) and mussels (W.NEUGREVE, personal communication). The renewed increase in the cadmium uptake values after the intermediate diminuation could be explained as uptake recurring as a result of the remaining available heavy metal content.

One possible explanation which I have evolved personally is the hypothesis of internal regulation and subsequent breakdown of the regulatory mechanism. The regulation can be regarded as an oscillatory process, involving initially an intense uptake which triggers a re-action in the form of a reduction in the cadmium concentration, with convergence towards an ideal value. Such an "overshooting" has been described by UEDA & SUZUKI (1976). If this ideal value, which is established by the high concentration of the toxic heavy metal in the medium, cannot be maintained, then the tolerance threshold is overstepped with the consequence that the regulatory system breaks down and then the uptake of the element once again begins.

THURBERG et al (1973) and JONES (1975) observed a cadmium-induced hypertonia in decapods and isopods. It is conceivable that the hypertonia is induced by an increase in the osmotic activity, which in turn leads to increased bioconcentration of cadmium (see 3.1.1.2). Such a sequence could cause a breakdown in the regulatory mechanism once the element is incorporated biologically.

Under the given experimental conditions, any attack on the cadmium species by the organic excretions of the crustaceans ought to be relegated to minor importance. The adsorption capacity of the sediment and its organic components exceeds by far the binding tendency of any excretions. On the other hand, an accumulation pattern displaying the kinetics of a dampened oscillation as a result of a regulatory mechanism operating for an initial period and then breaking down cannot be excluded. It is questionable, however, whether one can postulate a regulatory mechanism operating over a concentration range of three orders of magnitude if this level of cadmium cannot be kept below the tolerance threshold. Moreover, one must also conclude that the effect of higher cadmium concentrations would be to speed up the breakdown of the regulatory process. Such was not the case. If one assumes that the cadmium is not available for uptake as a result of the reduced concentration in the water, then it is highly unlikely that a renewed uptake could begin again as a result of the hypothesized regulatory mechanism when the cadmium concentration in the water is still decreasing. 123

In the experiments involving higher cadmium concentrations (greater than 1 mg Cd/L), mucus formation was observed. As an additional possibility for a physiologically-oriented accumulation pattern, the following mechanism comes into consideration: under the influence of the sudden cadmium stress, a mucus secretion occurs in the gills (GARDNER & YEVICH 1970; VERNBERG & O'HARA 1972; O'HARA 1973; THURBERG et al 1973; VOYER 1975; VOYER et al 1975). By excreting the cadmium-containing mucus, the crustacean reduces the level of the toxic heavy metal; subsequently the cadmium uptake begins once again at the reduced cadmium concentration in the water. Repetition of the mucus secretion does not recur since this time the concentrations are not sufficiently high to trigger this acute response to tissue damage.

The accumulation pattern was not affected at lower levels of cadmium concentration since no mucus formation was triggered.

Re b): In the studies involving administration of the cadmium in a single dose at the start of the experiment, the crustaceans were exposed to a very high level of cadmium stress right at the beginning. Within about 14 days the concentration of the element in the water sank by 2 to 3 orders of magnitude and reached a concentration plateau which

remained virtually constant until the end of the experiment. On the basis of knowledge about accumulation mechanisms, it can be assumed that Corophium has a very strong cadmium uptake initially. During the period in which bioconcentration was presumably occurring, the concentration in the water decreased with the consequence that a period of elimination was observed (predominantly de-sorption). Once a constant cadmium concentration has been established in the water, and after the adsorbed excess from the surface of the crustaceans has been removed, a slower bioconcentration process can begin.

Therefore it appears that the magnitude of the initial cadmium concentration does not affect the accumulation pattern, but only the level of the contamination. Verification of this finding is not possible since the data from the totality of the experiments were obtained from such a wide range of material that a statistical treatment would be very unreliable. It is clear, however, that the accumulation pattern is affected to a considerable extent by the rate at which the concentration in the water decreases. A rapid reduction in the cadmium concentration of the water can produce a cadmium elimination of 40% at the maximum. A slower reduction in the cadmium concentration in the water produced only a net standstill in the bioconcentration (see 3.1.3 exp.4&5). The hypothesis that the three-phase type of accumulation pattern is primarily affected by the method used to establish the cadmium concentrations at the beginning of the experiments has been confirmed by the results of the steady-state experiments (see below).

In the experiment in which cadmium was administered in repeated doses over the 14-day duration of the experiment, a continuous (inverse exponential) increase in the cadmium content of the crustaceans occurred (3.1.3no7).A graphical depiction of the accumulation pattern mirrors the profile of the first segment of a saturation function used to describe the accumulation pattern under the effect of constant cadmium concentrations in BENAYOUN et al (1974).

In detail, the accumulation pattern seems to be composed of 124
phases of alternately increased and reduced uptake, which correspond to the introduction of new dosages and concentration reductions in the water (see Fig.12, dotted line).

Under equilibrium conditions (cadmium distributed approximately equally between the water and the sediment, 3.1.3 exp.8), the accumulation pattern is linear over a wide range of concentrations (0-1.0 mg Cd/L in the system). Deviations from linearity appear to occur only sporadically at low cadmium concentrations during the initial phase of the accumulation. As was noticeable in the short-term experiments without sediment, variations in the rate of the accumulation-determining processes, namely adsorption and incorporation, may have produced an initially stronger adsorptive uptake of the element. Such deviations from linearity in the uptake process have also been recorded by DETHLEFSEN (1977), GILLESPIE et al (1977), and KLÖCKNER (1979). Both STENNER & NICKLESS (1974) and ZAROOGIAN & CHEER (1976) observed a linear increase in the cadmium content of mollusks at concentration levels up to a value of 5 µg Cd/L.

Therefore in the interpretation of the studies involving single doses administered at the beginning of the experiment, for the steady-state experiment with linear uptake one significant fact applies: the accumulation pattern of the type accumulation - elimination - accumulation is established by the sudden introduction of cadmium to the water followed by a subsequent reduction in the cadmium concentration, and not by any standard physiological reaction of the crustaceans.

The series of experiments using water of various salinities also fit into this picture. At salinities between 4% and 28%, as a result of the decreased cadmium concentration in the water the typical curve profile was always obtained; in addition, the accumulation was dependent upon the residual cadmium content of the water.

3.1.3.3 The significance of the food supply and the sediment characteristics for the bioconcentration of cadmium in *Corophium volutator*

In planning the experiments it was assumed that the availability of a nutrient-rich natural sediment which was contaminated would assure cadmium uptake from ingested material as well as from the water.

In the experiments up to 200 *Corophium* with a maximum weight of 800 mg were used. The natural mud flat sediment contained on the average

about one percent organically-bound carbon (Table 2). The available biomass therefore at the beginning of the experiment was about 20 g of carbon as a potential food source. As a result of aeration, an additional component from carbon dioxide was available. In all samples a continuous diatom formation was observed.

Although the generation cycles in Corophium volutator in the experiments corresponded to those in their natural habitat, and the increase in size of the juveniles seemed to be normal - 2 to 4 mm increase in length in a 30-day experiment - (reference orientation in the works of MORGAN 1965; McLUSKY 1967; GAMBLE 1970; FINCHAM 1971) the dry weight of the crustaceans decreased whereas the water content increased. (McLUSKY [1967] also observed an increase in size with the availability of an organically-rich sediment. However, he does not report any corresponding loss in weight for the crustaceans). In the course of a six-week experiment the water content rose from about 72% to approximately 82%. This phenomenon could not be prevented even by changing the sediment weekly, or by supplementing the food supply with dry feed (Tetramin). The water content of crustaceans in their natural habitat was also subject to fluctuation. However, the change in the water content of the crustaceans in the experiments did not correspond to the fluctuations in the natural habitat. Since a decrease in weight was always observed over the duration of the experiments, presumably it was caused by a reduction in the organic substrate of the sediment.

The extent to which heavy metals from the food supply undergo bioconcentration is a controversial subject. According to the concept of CEARLEY & COLEMAN (1973) the cadmium uptake from the food supply contributes more to the bioconcentration of the element than does the cadmium uptake from the water. BENAYOUN et al (1974) reported similar findings for Euphausiids.

On the other hand, SCHULTZ-BALDES (1973, 1974) reported that the bioconcentration of lead in mollusks was considerably less as a result of ingesting contaminated algae than from direct application to the mussels of a lead solution of the same concentration as that used to contaminate the algae. Our own experiments with limnetic mollusks and cyprinids confirm these results for the elements lead and cadmium (BIAS, in preparation). This would therefore correspond to the hypothesis

that inorganic heavy metal componds which are ingested solely through the food supply cannot produce a concentration in the consumer which is higher than the concentration in the food. An exception to this is the case of lipophilic heavy metal compounds which can be partially metabolized.

Numerous articles document the minimal rate of transfer of heavy metals to higher trophic levels (HOLDEN & TOPPING 1971; JERNELÖV & LANN 1971; PORTMANN 1971; LEATHERLAND et al 1973; PARRISH & CARR 1976; PATRICK & LOUTIT 1976; ZAUKE 1976). Analysis of invertebrates and teleosts of the same biotope as a general rule revealed higher heavy metal concentrations in the organism of the lower order (BOWEN 1966). Comparison of the cadmium content of omnivores and carnivores revealed no significant differences (MATHIS & KEVERN 1975).

It was therefore hypothesized that the introduction of a heavy metal through the food chain has less significant an effect on the bioconcentration level than does the direct uptake from water. This was confirmed by the Corophium experiments: the greatest uptake factors were observed at very low concentrations. This relationship is applicable in general to the process of bio-accumulation; it has been substantiated in numerous articles: for lead (SCHULTZ-BALDES 1972), mercury (KRAMER & NEIDHARDT 1975), iron (GEORGE et al 1976) and cadmium (KLÖCKNER 1979). Furthermore, in the Corophium experiments, the level of contamination of the crustaceans was dependent on the cadmium concentration in the water but not in the sediment. In the experiments with quartz sand (3.1.2 no.3) and with mud flat sediment (3.1.3 no.8 steady-state), for the same average concentration in the water, an uptake factor of 1000 was observed (relative to the fresh weight of the crustaceans) although by the end of the experiment saturation had not yet been reached. In 126
this test considerably more cadmium was adsorbed onto the nutrient-containing mud flat sediment than onto the quartz sand, but this difference had no visible effect on the uptake.

Analysis of the Corophium from the Elbe mud flats yielded cadmium levels between 0.1 and 0.15 µg Cd/g fresh weight. If an uptake factor of 1000 is applicable here as well (mean salinity 16‰), then the mean cadmium concentration of the water must be 0.1 to 0.15 µg Cd/L.

The actual analyses of the water (free of suspended particles) from the Elbe estuary at Otterndorf (ebb-tide) was 0.14-0.55 ug Cd/L. For comparison: FÖRSTNER & MÜLLER (1974) found values of 0.2 µg Cd/L at Brunsbuttel; BENAYOUN et al (1974) calculated from the water analysis data of HUYNH HGOC (1973), 0.1 µg Cd/L, (Mediterranean, Monaco) an uptake factor of 1500 for Euphausiids in those waters.

Since the same uptake factors were found for organisms in their natural environment as for the crustaceans in the experiments involving lower levels of cadmium concentration, despite the minimal or non-existent food supplements in the studies, this agreement supports the hypothesis that the uptake of the cadmium from the water is the primary source for the bioconcentration of the element.

Although Corophium is a sediment consumer, so that a close relationship was expected between the heavy metal content of the sediment and the bioconcentration in the amphipod, comparison of the uptake factors leads to the conclusion that the direct uptake and assimilation of the heavy metal from the sediment is of minimal importance. This interpretation agrees with the conclusions of CROSS (1970) and HALCROW et al (1973): no relationship between the metal content of the epifauna and the concentration in the associated sediment. CROSS (1968) and KRANZ (1979) used ^{65}Zn and ^{60}Co to establish that the uptake from the sediment was insignificant compared to the amount accumulated from the water.

The hypothesis that a considerable proportion of the cadmium bioconcentration results from the food supply has not been confirmed by these experiments. In fact there are indications that with a good food supply, the bioconcentration of cadmium in Corophium is reduced. In the experiment with the autoclaved sediment (3.1.3 no.3), the crustaceans suffered less weight loss than the organisms in the parallel experiment (3.1.3 no.2); despite the partially higher cadmium concentrations in the water, the crustaceans incorporated significantly less cadmium than those organisms in the parallel test. However, one ought to take into consideration the fact that the organic matrix may have reduced the availability of the cadmium. The amphipods removed from the mud flat in November were characterized by particularly low water content; under identical experimental conditions they tended to accumulate less cadmium than the higher water content crustaceans taken out in the spring.

These results fit into the spectrum of seasonal deviations in the heavy metal content of crustaceans as a reflection of their nutritional condition. In numerous studies of mollusks and crustaceans the highest heavy metal concentrations were always found after the winter break, namely at the conclusion of a period of low food supply; the lowest concentrations of heavy metals were found in the summer or autumn (BOYDEN 1974; FOWLER & OREGIONI 1976; KARBE et al 1975; PHILLIPS 1976; SCHNIER et al 1978). In order to explain this phenomenon, it is necessary to assume the consumption of stored food reserves, 127
thus introducing a relative concentrating effect on the toxic substances; however, it is also conceivable that the good nutritional condition of the organism precludes a toxic bioconcentration of the element, for example - hypothesis of McLUSKY (1967) - by adequate coverage from the food supply of the ionic requirements needed for the maintenance of a hypertonic hemolymph. An extensive bioconcentration is therefore prevented by osmotic activity. The reduced net accumulation in well-nourished organisms could thus be explained if the stored food reserves allow the toxic element to be first complexed and then partially excreted.

Crustaceans of various sizes were used in the experiments. To determine whether the variations in the bioconcentration results in the reproducibility tests were solely due to the differences in the vulnerability to contamination of the smaller organisms compared to the larger crustaceans, experiments were carried out to establish the relationship between uptake and average weight. These studies showed that there was a tendency for the smaller animals to have relatively higher cadmium concentrations than the larger crustaceans. However, within the size categories evaluated in the experiments this difference was insignificant. Crustaceans with a mean dry weight of 0.6-1.1 mg (length 5-7 mm) contained about 10% higher cadmium concentrations than those crustaceans with average dry weights of 1.5-2.0 mg (lengths 8-10 mm). Only those organisms with dry weights of 0.2-0.4 mg (lengths 2-4 mm) had significantly higher cadmium concentrations ($P<0.1\%$).

In all of the tables related to the accumulation pattern, the cadmium concentrations of the organisms are expressed relative to the corresponding dry weights. However, since the dry weights decreased in the experiments involving the natural sediment from the Elbe mud flats, and the water content of the organisms increased, it was necessary

to establish whether the uptake rates were affected by taking these weight losses into consideration.

Under the assumption that the amount of cadmium per individual is unaffected by the consumption of stored food reserves and thus remains constant, due to the decrease in dry weight the actual cadmium concentration in the organism increases. In order to differentiate this increase from the actual uptake of cadmium in the organism, it is necessary to subtract away that percentage increase in the cadmium concentration caused by the loss of dry weight. Due to the relatively large margin of error incorporated into the fresh weight determinations of such extremely small organisms, the mean weight loss encurred by all of the Corophium collected on one sampling day during the interval between consecutive samplings was used as the basis for the correction factor (Fig.15). In principle this factor had no effect on the uptake rates. Only in the second phase of the accumulation process was an asymptotic approach to the concentration-dependent saturation value visible in some of the experiments. For the linear accumulation under equilibrium conditions, incorporation of the correction factor into the calculation produced an attenuated increase and an uptake factor smaller by about 10% (maximum of 900 up to the end of the experiment). A noticeable deviation from linearity could not be discerned.

3.2.3 Discussion of the cadmium elimination in Corophium 134

In the experiments involving partial exchange of the water, it was astonishing how readily the sediment-bound cadmium was re-solubilized. Within a few days of a water change the previous equilibrium was re-established.

The rapidity with which the cadmium in the sediment was re-mobilized meant that although the amphipods excreted cadmium immediately following the dilution of the toxic heavy metal concentration in the water, just a few days later they began to accumulate cadmium again as the level in the water increased. Only if the crustaceans were transferred into an uncontaminated environment immediately following the bioconcentration phase did they effectively eliminate cadmium (30-50%).

Moreover, the excretion during the first 24-hour period proved to be more than half of the quantity eliminated over the entire duration of the experiment. From the high value initially for the cadmium content based upon the original weight of the crustacean (dry weight), the rate of elimination of the element decreased exponentially. After three weeks in an uncontaminated environment, no further cadmium was excreted by the crustacean; after six weeks the same values were obtained as after only fourteen days. Variations in the elimination pattern were associated with the duration of the accumulation process; there was a tendency for more cadmium to be eliminated following a short accumulation period relative to a longer uptake process.

Based upon these results it does not make sense to express the elimination of cadmium in terms of biological half-lives. RENFRO (1973), SMALL et al (1973), BENAYOUN et al (1974) and ADEMA (1976) have discussed the particular problems posed by the biological half-life, and indicate that in addition to the physiological differences, the choice of observation intervals also has an effect on the results. The critical factor here is the interaction of various mechanisms in the excretion of toxic substances. The elimination process is not only affected by the con- 135
centration interface between the organism and the medium, but also by a number of concentration gradients within the organism itself. The case of the crustaceans is complicated by the additional effect of eliminating discrete quantities of toxic substances through the moulting of exuviae. The methods of elimination which are involved here are: a) desorption, b) excretion and c) moulting of exuviae.

The desorption process only involves that fraction of the contaminant which is superficially bound to the external surfaces of the organism by adsorption and cationic exchange. The element ought to undergo desorption if the adsorption process occurred over an interval of several days, since incorporation of adsorbed cadmium into the interior of an organism requires a longer period of time (ROWE & MASSARO 1974). Apparently the metal is not yet metabolized even during the first phase of the accumulation process. In studies on rats CASTERLINE & YIP (1975) showed that following interperitoneal injection of cadmium chloride in physiological saline solution, bonding of the cadmium to protein began only after 24 hours had elapsed; as a rule the bonding occurred through

very specific sulfhydryl groups, from which it is very difficult to liberate the element (MARGOSHES & VALLEE 1957; KÄGI & VALLEE 1961; PULIDO et al 1966; NORDBERG et al 1972; RUGSTAD & NORSETH 1975; SUGAWARA & SUGAWARA 1975). Only the non-bound or loosely-associated component can be eliminated by desorption.

The fact that more complete elimination of cadmium occurs following a short period of accumulation relative to a longer period can be explained by the gradual conversion of the element to a more strongly-bonded species. A substantial proportion will still be subject to desorption. In elimination experiments with Nereis involving zinc, RENFRO (1972) also found that a shorter period of accumulation permitted a more effective elimination of that element.

It is still not clear whether the cadmium can still be excreted after metabolization to thioneines or other compounds. Compounds are produced having various molecular weights, as high as 6000 for a single polymer (CASTERLINE & YIP 1975; RUGSTAD & NORSETH 1975; SUGAWARA & SUGAWARA 1975); these tend to be stored and can produce an extreme bioconcentration of cadmium in the kidneys, liver and other analogous organs (SHUSTER & PRINGLE 1969; GARDNER & YEVICH 1970; JACKIM et al 1970; ROWE & MASSARO 1974; KING et al 1976; SETH et al 1976; SMITH et al 1976; WESTERNHAGEN et al 1978). The metabolization of cadmium represents a detoxification reaction in the opinion of several authors (WAGSTAFF 1973; RUGSTAD & NORSETH 1975; SUGAWARA & SUGAWARA 1975). During studies of cadmium proteins, only a small proportion of the compounds found could be dialyzed. Whether only this fraction can be potentially eliminated, or whether the stored compounds can also be excreted following further metabolization cannot be definitively answered at the present time. BENAYOUN et al (1974) recorded more than 50% elimination in Euphausiids within 6 days following a one-month accumulation period; within 96 days over 90% of the accumulated ^{109}Cd had been eliminated. The cadmium content of the faeces in this study was below the detection limit even in the period of greatest loss of cadmium. BOOTHE & KNAUER (1972) reported similar findings. Any elimination must 136
therefore occur through the removal of cadmium in the form of liquid excretions. Recent studies of limnetic mollusks and teleosts indicate

that a considerable fraction of the element cannot be eliminated at all (BIAS, in preparation).

In our own experiments, due to the deficiency of nutrient it was not possible to observe more than a few cases of adult crustaceans moulting their carapaces. Random sample snalyses showed that the cadmium content of the exuviae as well as the living organisms themselves corresponded to the dosages administered. Moulting of the carapace in the class of crustaceans is regarded as being a critaical detoxification mechanism (JOHNELS et al 1967; PEQUENAT et al 1969; FOWLER et al 1971; ARMSTRONG & HAMILTON 1973; LEATHERLAND et al 1973; ZAUKE 1977). BENAYOUN et al (1974) determined the cadmium content of Euphausiid exuviae, which correponded to a maximum loss of 18% at the beginning of the accumulation period and 5% four weeks later. The decrease in values over the duration of the experimental period suggests a gradient equilibrium established in peripheral tissues. The kinetics of elimination, and in particular the pronounced loss of cadmium immediately following the transfer to an uncontaminated environment, support the hypothesis that the primary method of elimination is desorption; this mechanism is obviously particularly important when the nutritional deficiency in the experiments limited both the moulting of carapaces and the excretion process. The results from the uptake experiments (strong elimination following a rapid decrease in the concentration of cadmium in the water) confirm this hypothesis.

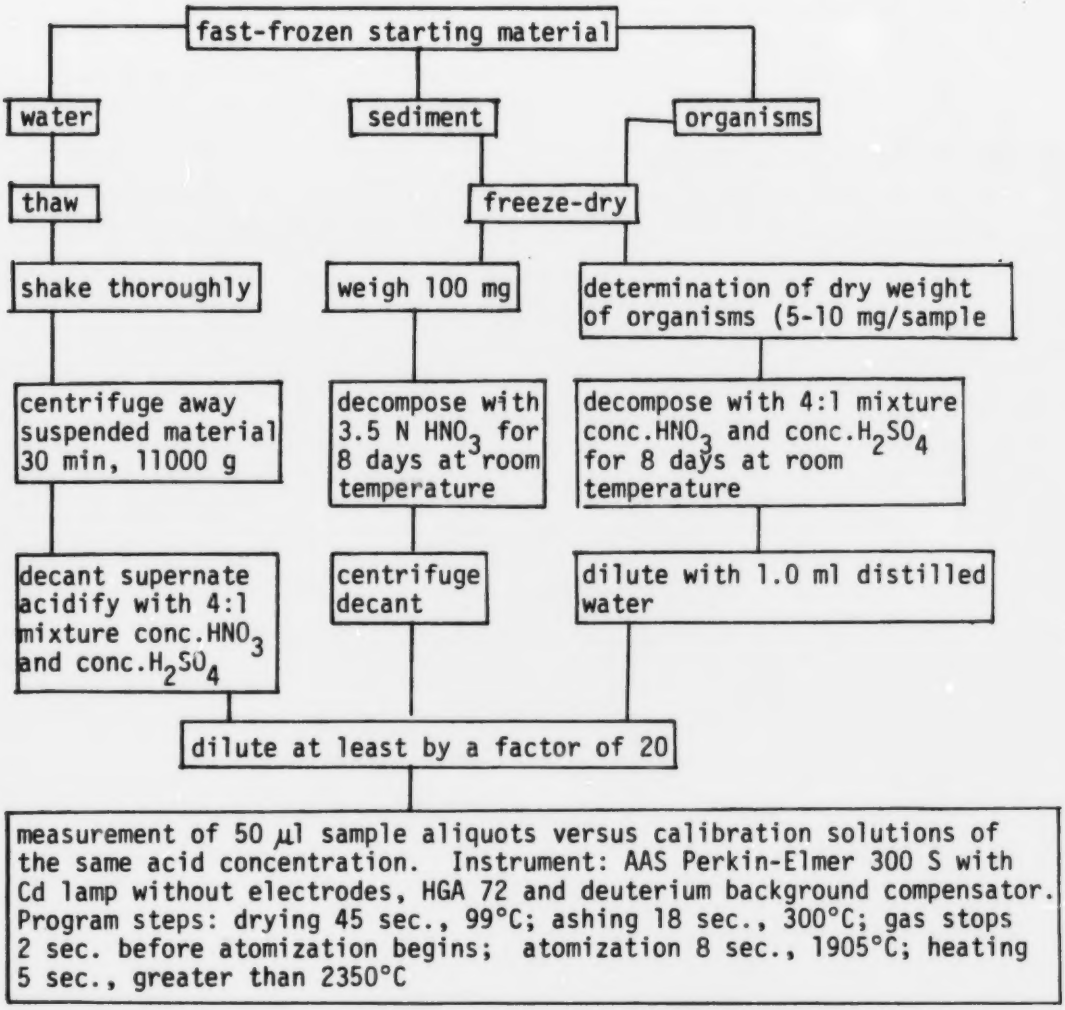
fast-frozen starting material
water
sediment
organisms
thaw
freeze-dry
shake thoroughly
weigh 100 mg
determination of dry weight of organisms (5-10 mg/sample
centrifuge away suspended material 30 min, 11000 g
decompose with 3.5 N HNO_3 for 8 days at room temperature
decompose with 4:1 mixture conc.HNO_3 and conc.H_2SO_4 for 8 days at room temperature
decant supernate acidify with 4:1 mixture conc.HNO_3 and conc.H_2SO_4
centrifuge decant
dilute with 1.0 ml distilled water
dilute at least by a factor of 20
measurement of 50 µl sample aliquots versus calibration solutions of the same acid concentration. Instrument: AAS Perkin-Elmer 300 S with Cd lamp without electrodes, HGA 72 and deuterium background compensator. Program steps: drying 45 sec., 99°C; ashing 18 sec., 300°C; gas stops 2 sec. before atomization begins; atomization 8 sec., 1905°C; heating 5 sec., greater than 2350°C

Fig.2 Sample preparation and analysis

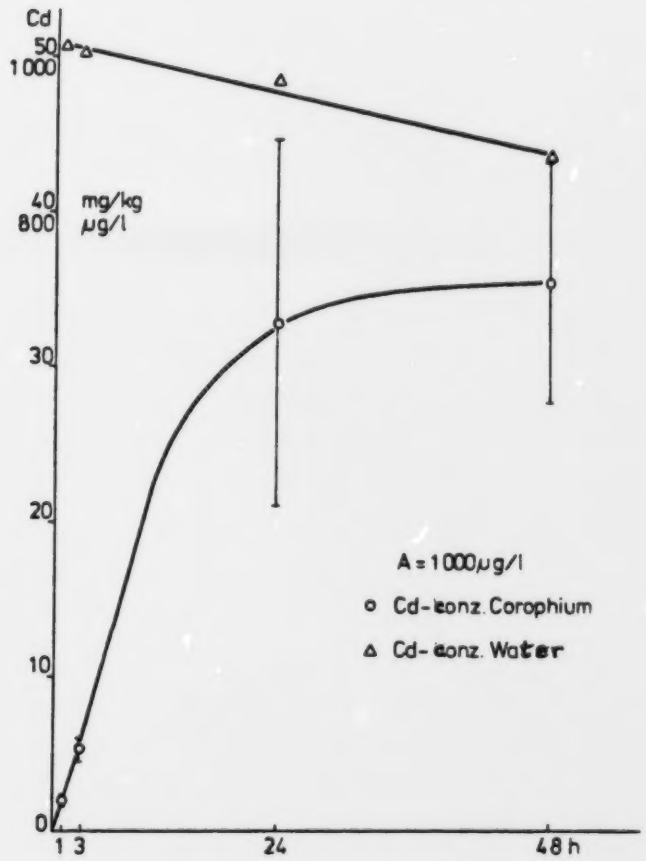

Fig.4 Cadmium accumulation pattern in short-term experiment. The graph shows the cadmium concentration development in water and in the organisms. The cadmium concentrations in the organisms are relative to their dry weights. The intervals shown give the standard deviations for the measurements, n=8. A = initial concentration, salinity of the water 16%.

Table 6 Cadmium accumulation in experiments with natural sediment and autoclaved sediment, salinity 16% (3.1.3 exp.2&3). All data are mean values from two duplicate samples. The values from the experiments with untreated and autoclaved mud flat sediment are reproduced here. The numbers in brackets come from samples in which more than 50% of the organisms died. The values preceded by an "X" are the cadmium values in the water after 56 days. 112

initial concentration (ug Cd/l)	cadmium content after _ days							
	1		4		11		35	
	no treat.	auto-clav.	no treat.	auto-clav.	no treat.	auto-clav.	no treat.	auto-clav.
300								
organisms	10.4	12.2	11.6	11.1	10.2	7.2	18.6	11.2
water	31	36	17	15	7.5	11	5.0	8.5
							X 3	
1000								
organisms	29.6	33.1	36.5	21.8	24.7	25.3	38.9	(31.5)
water	63	80	32	38	26	33	14	20
							X 9	
3000								
organisms	71.5	72.5	67.3	73.1	81.3	52.0	128	(68.3)
water	346	490	80	120	103	67	42	49
							X 51	
10000								
organisms	214	201	162	144	151	134	(296)	-
water	1720	3880	1100	890	481	175	86	64
							X 124	
controls								
org.(ug/g)	1.2	2.5	1.2	3.2	0.9	2.2	1.0	1.8
H_2O (ug/L)	1.2	1.5	1.2	<1	<1	<1	<1	<1
							X <1	

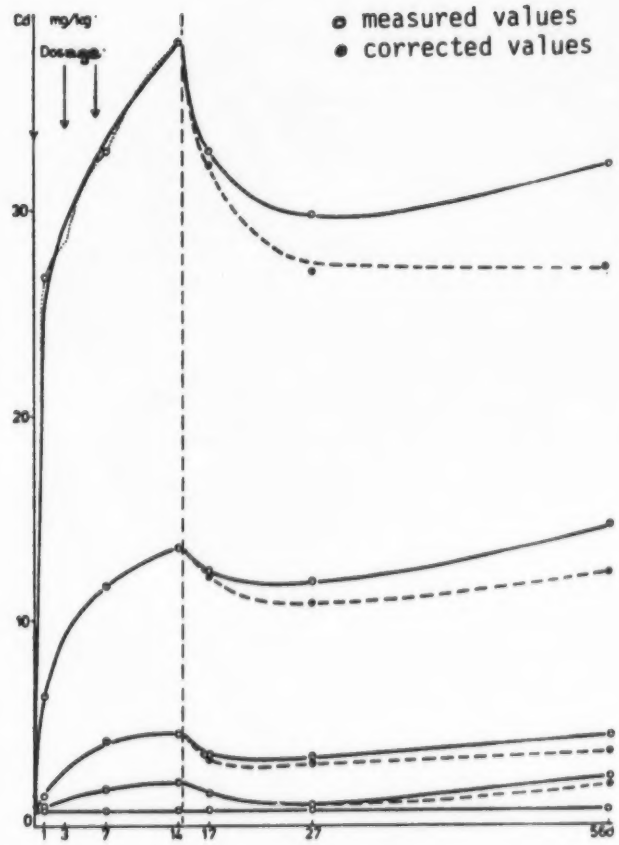

Fig.12 Cadmium accumulation after administration of several dosages (3.1.3 no.7) and cadmium elimination in an uncontaminated environment (3.2.2 no.1). The pattern for various concentrations is shown. The dotted line gives the hypothetical rate of uptake for the respective concentration. The dashed line gives the elimination incorporating the cadmium content of the crustaceans initially (dry weight) at the beginning of the elimination. For further explanations see the body of the text.

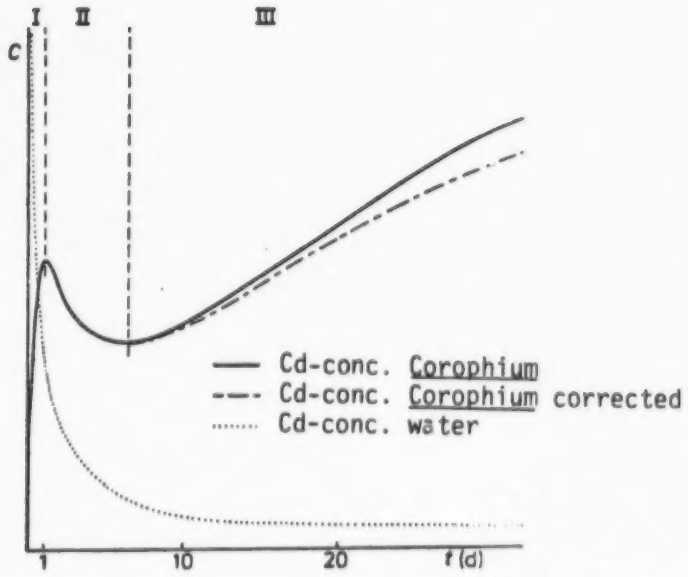

Fig.15 Schematic representation of the accumulation pattern with administration of cadmium dosage at the beginning of the experiment. The decrease of the cadmium concentration in the water is shown (dotted line) and the resulting accumulation pattern in Corophium volutator. The solid line shows the development of the true cadmium concentration in the organisms; the dot-dash line shows the actual content relative to the initial values in the organisms at the beginning of the experiment see text 3.1.3.3). The sections I, II and III indicate the 3 phases of the accumulation pattern.

Summary

Indications that cadmium has a long biological half-life and a high toxicity provided reason for an investigation of the kinetics governing the accumulation and elimination of this heavy metal. The aim of this study was to determine the distribution pattern of cadmium added intermittently to a system of water–sediment–organisms. The experimental conditions were varied (sediment type, salinity, cadmium concentration) in order to obtain some detailed information on the complex reaction in the accumulation processes following the introduction of cadmium into an ecosystem, such as the Elbe Estuary.

The accumulation in the test organism, *Corophium volutator*, kept in waters of different salinities, followed a linear curve for 92 hours at concentrations up to 30 µg Cd/l. At higher concentrations, the corresponding accumulation rates decreased with time, indicating that saturation was being approached. The salinity influenced the accumulation rate. At identical cadmium concentrations the resulting accumulation was greater at lower salinities (c. 4‰) than at higher values (up to 28‰ S). The ratio of the accumulation rates at 4‰ and at 28‰ S is 4 : 1.

In long-term experiments with quartz-sand sediment of known particle size, the adsorption capacity of the fine-grain sediment (63—125 µm) proved to be superior to that of coarse-grain sand and gravels (250—500 µm). Substantial reduction of the cadmium concentration due to adsorption resulted in an intermittent reduction of the cadmium-accumulation rate in *Corophium*. (At initial concentrations of 3.0 mg Cd/l in the system and volume ratios, water to sediment, of 3.6 : 1.0, the amount removed from the water by sediment of 63—125 µm particle size equalled 60—80%.) Natural sediment from the Elbe mud flats, under similar conditions, effected a decrease of cadmium concentrations in the water by two or three orders of magnitude. The adsorption of cadmium by the sediment was greater at low (4‰ S) than at high salinity (~ 28‰ S).

The accumulation of cadmium in *Corophium volutator* proved to be predominantly dependent on the concentration in the water. The sediment-bound cadmium seemed to be unavailable, or not readily available, to the test animals.

In parallel tests with the same amount of cadmium per system, salinities were maintained at various values from 4 to 20‰. The concentration in all test animals was of the same magnitude. The greater adsorption by the sediment a low salinity is fully compensated for by a more intensive accumulation in the organisms.

In the experiments with mud-flat sediment, cadmium was usually administered as a single dose after the test animals had become adapted to the experimental habitat. Under these conditions, a characteristic three-phase accumulation pattern was observed:

1. At the beginning, a steep increase in cadmium content, lasting from 24 hours to 4 days, results from high concentration. During this time the rate of accumulation decreases.
2. Over a period of 4 to 8, and sometimes as much as 14 days, the elimination rate exceeds the accumulation rate until the concentration in the water approaches a constant value.
3. Accumulation resumes, leading to an increasing cadmium content in the animals over a long period of time (6 weeks to 3 months). The rate levels off gradually.

However, when the crustaceans were introduced after the cadmium had established an equilibrium in the system (4 weeks after dosage) the resulting accumulation was linear over the entire experimental period of 43 days. Therefore the three-phase accumulation pattern is interpreted as the organisms' reaction to the changes in the cadmium concentration in the water.

Application of discrete quantities of cadmium at the beginning of a test, or, alternatively, in several portions at intervals of a few days, did not bring about significantly different accumulation values in the *Corophium*.

In elimination experiments, the water containing cadmium was exchanged for uncontaminated water. After single or multiple water changes, the original cadmium concentrations, present before water renewal, were reestablished within a few days due to leaching from the sediment. Thus, following each water change, elimination of cadmium occurred only for a short time. Accumulation in the organisms continued as the concentration in the water rose.

Regardless of the conditions during accumulation (with or without sediment), the elimination in uncontaminated media never exceeded 50% of the total absorbed. The most significant loss of cadmium occurred only during the first seven days. Desorption is the most important mechanism of elimination.

At optimum salinity (16‰) in experiments with and without a nutritional source, concentration factors of 900—1000 were obtained. These are in the same order of magnitude as for the *Corophium* population in the Elbe Estuary, calculated on the basis of water analyses to be 0.14—0.56 µg Cd/l. From this comparison, the conclusion can be drawn that cadmium accumulation from ingested material is of secondary importance in the natural habitat as well.

Differences in accumulation by adult amphiphods according to their size could be neglected. In contrast, juvenile animals accumulated significantly higher concentrations than adults. No significant differences in cadmium concentrations were found when males and females were compared.

Large amounts of cadmium resulting from posthumous intensive uptake, as repeatedly reported in literature, could not be detected in *Corophium*. Analyses of dead but still undecomposed animals revealed cadmium concentrations similar to those in living specimens.

Toxicological phenomena could be already observed at concentrations in the water of about 10 µg Cd/l. The crustaceans exhibited increased irritability. On the other hand, concentrations above 30 µg Cd/l caused a depressed reactivity. At concentrations greater than 100 µg Cd/l mortality increased.

Ecological implications, affecting the intertidal mud flats, could be expected from an extended recycling of cadmium because of its high mobility at the water-sediment interface. Another potential hazard involves peak concentrations, even if these are of short duration. Since the shrimps have only a limited capabilty to eliminate cadmium, high concentrations would remain in their tissues long after even a short-term exposure.

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